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Serum vibriocidal responses when second doses of oral cholera vaccine are delayed 6 months in Zambia

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ABSTRACT

Two-dose killed oral cholera vaccines (OCV) are currently being used widely to control cholera. The standard dose-interval for OCV is 2 weeks; however, during emergency use of the vaccine, it may be more appropriate to use the available doses to quickly give a single dose to more people and give a delayed second dose when more vaccine becomes available.

This study is an open label, randomized, phase 2 clinical trial of the vibriocidal response induced by OCV, comparing the responses when the second dose was given either 2 weeks (standard dose interval) or 6 months (extended dose interval) after the first dose. Vaccine was administered to healthy participants > 1 year of age living in the Lukanga Swamps area of Zambia. Three age cohorts (<5 years, 5–14 years, and ≥ 15 years) were randomized to the either dose-interval. The primary outcome was the vibriocidal GMT 14 days after the second dose.

156 of 172 subjects enrolled in the study were included in this analysis. The Inaba vibriocidal titers were not significantly different 14 days post dose two for a standard dose-interval GMT: 45.6 (32–64.9), as compared to the GMT 47.6 (32.6–69.3), for the extended dose-interval, (p = 0.87). However, the Ogawa vibriocidal GMTs were significantly higher 14 days post dose two for the extended-dose interval at 87.6 (58.9–130.4) compared to the standard dose-interval group at 49.7 (34.1–72.3), p = 0.04. Vibriocidal seroconversion rates (a > 4-fold rise in vibriocidal titer) were not significantly different between dose-interval groups.

This study demonstrated that vibriocidal titers 14 days after a second dose when given at an extended\ dose interval were similar to the standard dose-interval. The findings suggest that a flexible dosing schedule may be considered when epidemiologically appropriate.

The trial was registered at Clinical Trials.gov (NCT03373669).

1. Introduction

Over a billion people are at risk for cholera globally and the region reporting the largest number of cases is sub-Saharan Africa [1,2]. Cholera is transmitted via fecal-oral transmission, primarily through contamination of drinking water with toxigenic *V. cholerae*; [3] hence, the disease occurs more frequently in areas with poor hygiene and sanitation [4]. Improving access to clean water,

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sanitation and hygiene in concert with enhanced surveillance, promoting health education and vaccination are critical interventions for comprehensive cholera prevention and control [5]. However, achieving such measures remains a challenge in the resource-constrained regions of sub-Saharan Africa, Asia and Hispaniola where cholera burden is the greatest [1,2].

There are currently two identical killed whole cell bivalent (O1 and O139) Oral Cholera Vaccines (OCV) prequalified by the World Health Organization (WHO) and available through the global stockpile: Shanchol (Shantha Biotechnics Limited, India), and Euvichol (Eubiologics, South Korea). Both package inserts state that the vaccine is to be given in a 2-dose regimen with the second dose being given approximately 2 weeks after the first [6]. This dose interval, which was used during the pivotal field trial, [7] was based on several assumptions, including: a) a previous understanding that two doses were needed to confer the highest levels of immunity [8], b) that vaccine would generally be given to control an outbreak, so two doses need to be administered as quickly as possible, and c) one should avoid giving doses too close together to achieve boosting from the second dose. However, additional experience with OCV suggests that these assumptions may not be accurate, and a second dose could be given later without sacrificing effectiveness.

Recently published findings suggesting that the second dose may be given later include the following. Firstly, a single dose does stimulate protection for several months, for at least one year in subjects over 5 years of age [9]. Vaccine efficacy following a single dose was 40% against all cholera episodes and 63% against severely dehydrating cholera episodes; thus, reducing the urgency to provide the second dose quickly during an outbreak. Secondly, due to limited supply of vaccine, models of OCV use during an outbreak show that more cholera cases will be prevented if available doses are used to vaccinate more people with a single dose rather than fewer people receiving two doses [10]. Finally, serological studies found that a second dose given 14 days after the first does not increase the vibriocidal geometric mean titer (GMT) or the seroconversion rate [11,12]. Also rates of seroconversion were similar when comparing a 2-week dose interval versus seroconversion at a 4-week dose interval [12]. These findings suggest that a more flexible vaccine dosing schedule may not be detrimental to the vaccine immunogenicity.

The primary aim of this project was to compare the vibriocidal GMTs 14 days (14D) post second OCV dose among participants randomized to the standard 2-week dose-interval group (DIG1) versus participants randomized to the extended, six-month dose-interval group (DIG2). We hypothesized that the vibriocidal GMT for DIG1 14D post dose 2, when given at an extended 6-month dosing interval, will not be inferior to the vibriocidal GMT 14D post dose 2 when given according to the standard dose interval. The secondary aims included a) assessment of vibriocidal antibody response rates 14D post dose 2 among subjects in DIG1 versus 14D post dose 2 among participants in DIG2, b) determination of age-specific serum vibriocidal GMTs during the same time intervals, and c) persistence of elevated vibriocidal GMTs after longer periods following vaccination in both groups.

2. Materials and methods

2.1. Study design and participants population.

This was an open label, randomized, phase 2 clinical trial of the vibriocidal responses following administration of OCV to participants in three age cohorts (<5 years, 5–14 years, ≥15 years). Vaccine dosing and follow-up visits were completed between November 2017 and August 2018. All participants were residents of the Lukanga Swamps area, located 70 km northwest of Kabwe and 130 km from Kapiri-Mposhi districts of central Zambia. The Swamps fall within the Waya Health Centre catchment area serving a population of 21,000, with 16,000 living on the upper land [13]. Participants eligible for inclusion in this study included healthy individuals>1 year of age, living in the catchment area and available for the duration of the study. Exclusion criteria included: the presence of a significant medical or psychiatric condition (Example: renal insufficiency hepatic disease), pregnancy (determined by urine pregnancy test), diarrhea within the previous 7 days or a history of chronic diarrhea (defined as ≥ 3 unformed loose stools in 24 h), ever having received an OCV, and receipt of an investigational drug product (within 30 days before vaccination).

2.2. Randomization

Participants were randomized into two dose-interval groups (DIG) in a 1:1 ratio using a computer-generated algorithm: subjects in DIG1 received the second dose 14D post dose 1 while subjects in DIG2 received the second dose 6 M post dose 1. Blood samples were collected at strategic time points at baseline and throughout the study (Fig. 1). The study used block randomization, with 6 persons per block. Within each of the three age strata, a computer-randomized list of DIG assignments was prepared, with equal numbers per age group randomized per arm. Eligible participants gave informed consent and were subsequently enrolled in the study.

The vaccine used in the study was an oral cholera vaccine [14,15] which consists of a mixture of killed *V. cholerae* O1 and O139 (Shanchol Lot #'s SCN019A15 and SCN015A16). Participants were instructed not to eat 1 h before and 30 min after taking the vaccine. The vaccine vial was thoroughly mixed and inspected by the study physician prior to administration to the participant. To assure safety, all participants were observed for 30 min after taking the vaccine. All participants were contacted by the study coordinator daily for three days to detect illnesses after receiving each dose of the vaccine.

2.3. Laboratory procedures

Venipuncture was performed, and serum samples were separated from whole blood. Serum was transported at 2 – 8®C and stored at – 20 °C at the Centre for Infectious Diseases Research in Zambia (CIDRZ) laboratory, Lusaka, Zambia until vibriocidal antibody assays were performed at the CIDRZ laboratory. The vibriocidal titers were determined using previously described methods [15]. Zambian *V. cholerae* strains (Inaba -EDVRU/ZM/09–10 and Ogawa EDVRU/ZM/2016) were used as the target vibrio strains for these assays in Zambia. Preliminary studies at Johns Hopkins University demonstrated that assays performed using these strains from Zambia were comparable to results using strains Inaba (T19479) and Ogawa (X25049 [16] Briefly, the strains were incubated with dilutions of heat-inactivated serum and exogenous guinea pig complement at 37^{O}C for 1 h, shaking (50 rev/min). Vibriocidal titers were defined as the reciprocal of the highest serum dilution resulting in a 50% reduction in optical density (595 nm) compared to controls without serum. A standard O-antigen specific monoclonal antibody (mAb) [17] and a high titer standard serum were used to normalize the results in case of -inter-assay variations [18]. Seroconversion was defined as a 4-fold or greater increase in vibriocidal titers from the baseline (D0) titers.

2.4. Statistical methods

The vibriocidal titers were determined for each subject and the GMTs of the sera for each time point for all age groups as well as each age cohort were determined. The primary analysis was per

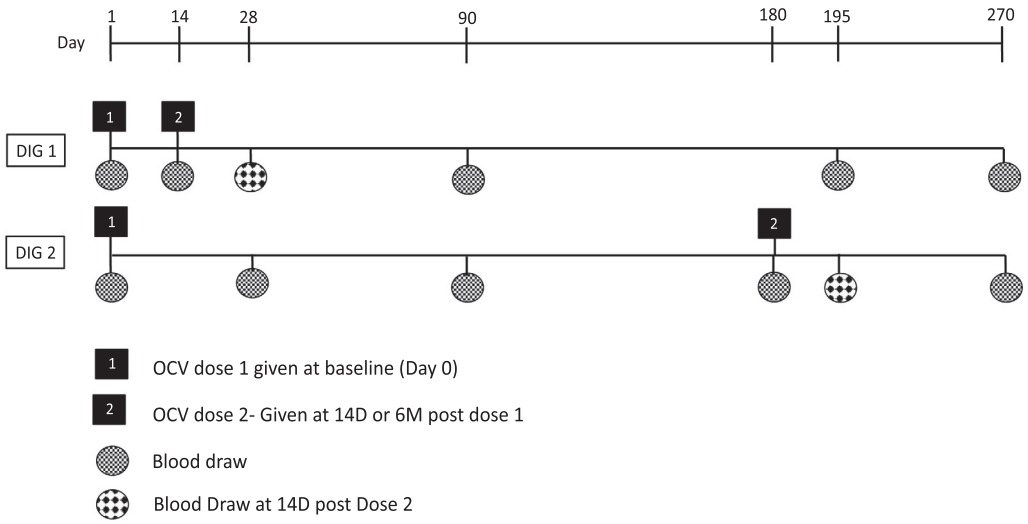

Fig. 1. Timeline for blood draw and vaccination. Blood was collected at six different time points for both DIG 1 and 2. Vaccine was administered at D 1 and 14 for DIG 1 while for DIG 2; it was at D 1 and 180. **DIG** = Dose Interval Group, **D** = Day.

protocol and compared the vibriocidal GMT for group DIG1 versus DIG2 14 days after the second dose. Secondary analyses included 1) comparison of vibriocidal seroconversion rates 2 weeks after the second dose between DIG1 and DIG2, 2) comparison of age group-specific vibriocidal GMTs and seroconversion rates 14 days after the second dose between DIG1 and DIG2. Additional exploratory analyses compared persistence of antibody titers at time points after the two-week interval.

The sample size was calculated to compare the difference in two GMTs sufficient to demonstrate a difference in the vibriocidal GMT for the two groups 14 days after the second dose. The margin of non-inferiority was set to 15%. The true difference between the two GMTs was assumed to be 0.000. The data assumed a population with a standard deviation of 7.770 for both groups. With alpha = 0.025, beta 0.10, one-sided, two-sample mean, and using the scoring method of non-inferiority test, a sample size of 11 in each group was required. Assuming a 10% high baseline titer and 20% dropout over the study period, a sample size of 20 per age-strata was calculated, totaling 120 subjects in the study. Additional subjects were included to adjust for dropouts over the prolonged period of follow-up.

For comparison of the GMTs, a Student's *t*-test was performed on GMT's logarithm scale using either the pooled or Satterthwaite method depending on whether the variances were equal or not. Non-parametric bootstrap of 100,000 iterations was used to estimate the 95% confidence interval of the GMT ratio. Analyses for comparisons of dichotomous outcomes of seroconversion were performed with the Chi-square test (or Fisher's exact test). Baseline variables were compared between the two groups using Student's *t*-test for continuous variables and Chi-square test(or Fisher's exact test) for categorical variables. Statistical evaluations of all comparisons were two-tailed. Stata/SE 14.0 [College Station, Texas] was used to analyze the data.

2.5. Ethical Approval

The study was approved by the Institutional Review Board at Johns Hopkins University (IRB00008066) and by the University of Zambia Biomedical Research Ethics Committee (UNZABREC-REF No. 002–10-17). Potential study participants were given detailed information about the study and screened for eligibility by the study physician. Informed consent was obtained for all adults, as well as parents or guardians of participating children. Written assent was provided by participants aged 12–17 years. The trial was monitored by local monitors and was registered at Clinical Trials.gov (NCT033736699).

3. Results

3.1. Enrollment

From November 2017 – August 2018, 174 participants were screened and 172 were enrolled in the study. Two participants were excluded, one had chronic diarrhea and one was pregnant. The 172 remaining participants were randomized to DIG1 (87) and DIG2 (85) as shown in Fig. 2. The demographic characteristics of the study participants were similar between the two dose-interval groups (Table 1).

All participants in DIG1 received both doses. Among participants in DIG2, 84/85 (98.8%) received the first dose and 79/85 (93%) received the second dose, respectively. Blood draws were not completed for 7 participants (5 = DIG1 and 2 = DIG2) on D0 (baseline) and thus, they were excluded from the analysis. In addition, 9 participants were lost to follow-up and 17 participants missed $\geq$ 1 follow-up visit. One hundred and fifty-six participants were included in the final analysis. The consort flow diagram of the study is shown in Fig. 2.

3.2. Immunogenicity data

The baseline vibriocidal GMTs for participants in DIG1 and DIG2 for both Inaba and Ogawa serotypes were similar as shown in Table 2, but as expected, the GMT increased on D14 for DIG1. Although a D14 serum sample was obtained only from subjects in DIG1, we assume the GMT on D14 is similar to DIG2 since the two groups were randomized. For DIG1, the GMT on D28 (14D post dose 2 for DIG1) was similar to the titer 14D post dose 1; it did not increase further as a result of the second dose. The GMT was, however, higher for DIG 1 on D28 compared to DIG2 (4 weeks post dose1) suggesting the second dose on D14 for DIG1 extended the increased GMT titer through at least D28. By D90, the GMTs were similar comparing the two DIGs. As expected, when the second dose was administered to DIG2 6 months later, this stimulated an increase in vibriocidal GMT. Of note, the GMTs at all follow-up time points for both DIG1 and DIG2 were higher than the baseline GMT.

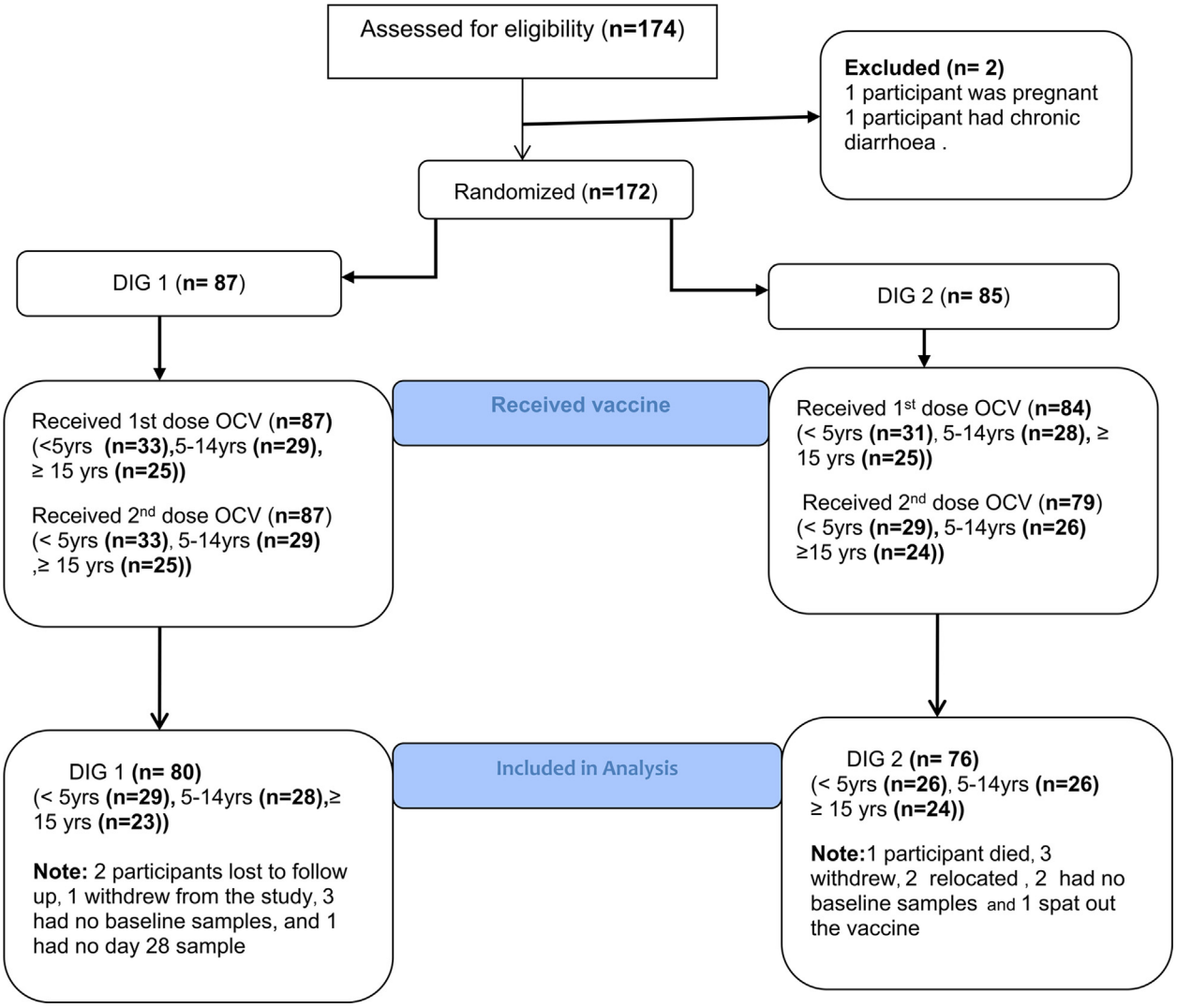

Fig. 2. Consort chart. Children and adult subjects enrolled in the study. They were 156 participants who met the criteria for analysis.

Fig. 3 shows the overall trend of the GMT serum titers over time by serotype. As shown, the GMTs increased following administration of a dose to each group. The results of the primary outcome, as shown in Table 3 and supplement (Figure S1), demonstrate that the vibriocidal GMTs 14D post dose 2 were not different for the Inaba serotype; however, the GMT was higher for Ogawa for DIG2: 87.6 (95% CI: 58.9–130.4) as compared to the GMT for DIG1: 49.7 (95% CI: 34.1–72.3) (p-value of 0.04). Similarly, when evaluating the Geometric Mean Titer Ratio (GMR) 14 days after the second dose comparing DIG2 to DIG1, GMR for Inaba was 1.04 (95% CI: 0.63–1.73) and for Ogawa was 1.76 (95% CI: 1.03–3.01).

The GMTs 14D post dose 2, stratified by age group, is shown in Fig. 4. The GMTs for DIG2 were slightly, but not significantly, higher for age group < 5 and for those ≥ 15 years. The GMTs were higher for participants aged 5 – 14 years for both DIG1 and DIG2 for both serotypes.As shown in the Supplement (Figure S2), the seroconversion rates 14D post dose 2 were similar for the entire group, as well as the age-stratified groups.

4. Discussion

This study in a rural area of Zambia found that the serum vibriocidal GMT and response rates following a second dose were similar when comparing the standard or an extended dose-intervalof 6 months. Although the Ogawa vibriocidal GMT titer was higher in the extended dose, DIG2 group, the overall findings suggest that the titers were generally very similar, and the vibriocidal GMTs were not different when given at six months compared to the standard two week dose interval. The vibriocidal GMTs quickly increased following the first dose as shown in DIG1, but the second dose did not result in a further increase. The titer on D28 for DIG1, 14D post dose 2, was higher than DIG2, which had received only one dose by that time, suggesting that the second dose on day 14 prolonged the elevated titer. However, this difference did not persist when measured three months later. Thus, the elevated titer was not sustained.

Similarly, the second dose given to DIG2 at 6 months stimulated a brisk rise in GMT, but this also fell resulting in similar GMTs between the groups three months post dose 2. It should be noted that all follow-up GMTs were elevated in comparison to the baseline GMT. There appeared to be no major differences by age group, however, age group 5–14 years may have responded with a somewhat higher titer in comparison to the other two age groups.

We hypothesized that an extended dose-interval of six months (DIG2) would not be inferior to the standard dose interval of two weeks, (DIG1). When considering the predefined non-inferiority margin of 15% used to determine our sample size, we restricted our results to an extremely strict margin (0.85–1.17). Our study results support non-inferiority for an extended dose interval

Table 1
Demographic features of Participants.

		14-day interval DIG1 (N = 87)	6-Month Interval DIG2 (N = 85)	P value
Age in yrs (mean (SD))		13.3 ± 14.3	13.0 ± 14.2	0.88
Age group (n (%))	1–4	33(37.9%)	32(36.7%)	0.99
	5–14	29(33.3%)	28(32.9%)	
	>14	25(28.8%)	25(29.4%)	
Male (n (%))		38(43.7%)	38(44.7%)	0.89
Average weight(kg) by age group (mean(SD))	1–4	11.75 (2.77)	11.38 (3.13)	0.60
	5–14	28.55 (13.41)	25.54 (9.49)	0.33
	>14	56.00 (12.10)	56.44 (11.41)	0.90
Average height(m) by age group (mean(SD))	1–4	0.86 (0.14)	0.86 (0.13)	0.93
	5–14	1.28 (0.19)	1.21 (0.13)	0.15
	>14	1.58 (0.13)	1.59 (0.09)	0.78
Z score for the children $\leq$ 4 (mean (SD))	Weight-for-age	−1.51 (3.27)	−1.44 (2.26)	0.91
	Height-for-age	−0.94 (1.80)	−1.19 (1.22)	0.52
BMI (kg/m^2) for those > 4 (mean(SD))		19.91(7.04)	19.58(5.58)	0.79
Education for those > 14 years (n (%))	No schooling	4(16%)	7(28%)	0.46
	Primary school	14(56%)	10(40%)	
	Secondary school	7(28%)	8 (32%)	
	Above secondary school	0	0	
Father's education (n (%))	No schooling	26(29.9%)	37(43.5%)	0.11
	Primary school	29(33.3%)	26(30.6%)	
	Secondary school	29(33.3%)	22(25.9%)	
	Above secondary school	3(3.5%)	0	
Mother's education (n (%))	No schooling	29(33.3%)	38(44.7%)	0.25
	Primary school	37(42.5%)	33(38.8%)	
	Secondary school	21(24.2%)	14(16.5%)	
	Above secondary school	0	0	
House ownership (n (%))		78 (89.7%)	82(96.5%)	0.08
Main material of flooring in the house (n (%))	Tiles	0	0	0.61
	Cement/Brick	10(11.5)	12(14.1)	
	Mud/Dirt	77(88.5)	73(85.9)	
	Other	0	0	
Main source of drinking water (n (%))	Own Piped/Borehole/Tube well	11(12.6)	11(12.9)	0.28
	Piped/Borehole/Tube well from neighbor	50(57.5)	42(49.4)	
	Piped/Borehole/Tube well far from home	2(2.3%)	0	
	Unprotected Well/Pond/Canal	24 (27.6%)	32 (37.7%)	
	Other	0	0	
Type of toilet facility (n (%))	Open Pit Latrine	22 (25.3%)	28 (32.9%)	0.22
	Covered Pit latrine	63 (72.4%)	57 (67.1%)	
	Flush toilet	0	0	
	Open Defecation (on the ground)	2 (2.3%)	0	
	Open Defecation (in the river)	0	0	
	Other	0	0	

Table 2
Comparison *V. cholerae* 01 Inaba and Ogawa baseline titers by age group.

Inaba									
Baseline titers	**DIG1 n = 31 (%)**	**DIG2 n = 27 (%)**	**P value**	**DIG1 n = 27 (%)**	**DIG2 n = 26 (%)**	**P value**	**DIG1 n = 22 (%)**	**DIG2 n = 23 (%)**	**P value**
<10	26 (83.9)	24 (88.9)	0.85	14 (51.9)	18 (69.2)	0.37	16 (72.7)	15 (65.2)	0.99
10–40	2 (6.4)	2 (7.4)		9 (33.3)	4 (15.4)		4 (18.2)	5 (21.7)	
>80	3 (9.7)	1 (3.7)		4 (14.8)	4 (15.4)		2 (9.1)	3 (13.1)	
Ogawa									
1–4				**5–14**			**>14**		
Baseline titers	**DIG1 n = 31 (%)**	**DIG2 n = 27 (%)**	**P value**	**DIG1 n = 27 (%)**	**DIG2 n = 26 (%)**	**P value**	**DIG1 n = 22 (%)**	**DIG2 n = 23 (%)**	**P value**
<10	27 (87.0)	21 (77.8)	0.67	11 (40.7)	16 (61.5)	0.06	15 (68.2)	10 (43.5)	0.12
10–40	2 (6.5)	4 (14.8)		11(40.7)	3 (11.5)		5 (22.7)	5 (21.7)	
>80	2 (6.5)	2 (7.4)		5 (18.5)	7 (26.9)		2 (9.1)	8 (34.8)	

(DIG1) compared to the standard dose interval, (DIG1) for Ogawa serotype (lower bound of 95% CI 1.03 > 0.85). However, the results of Inaba do not support non-inferiority (lower bound of 95% CI 0.63 < 0.85). However, it is well established that most non-inferiority vaccine RCTs use a GMT ratio of 1.5 or 2 [19]. Applying the commonly accepted ratio of 0.5/2; our study demonstrates that an extended dose-interval of six months is not-inferior to the standard dose interval for Ogawa (1.03 > 0.5) and Inaba (0.63 > 0.5).

This study is the first to evaluate the vibriocidal responses after a prolonged dose-interval and provides additional information regarding the duration of elevated titers following OCV. These findings are consistent with findings from Kolkata in which the vibriocidal titers were compared when OCV was given at the standard 2-week versus a 4-week dose-interval [12]. The Kolkata study, as well as a study from South Sudan [20] observed a lack of further increase in GMT when the second dose was given 2 to 3 weeks post

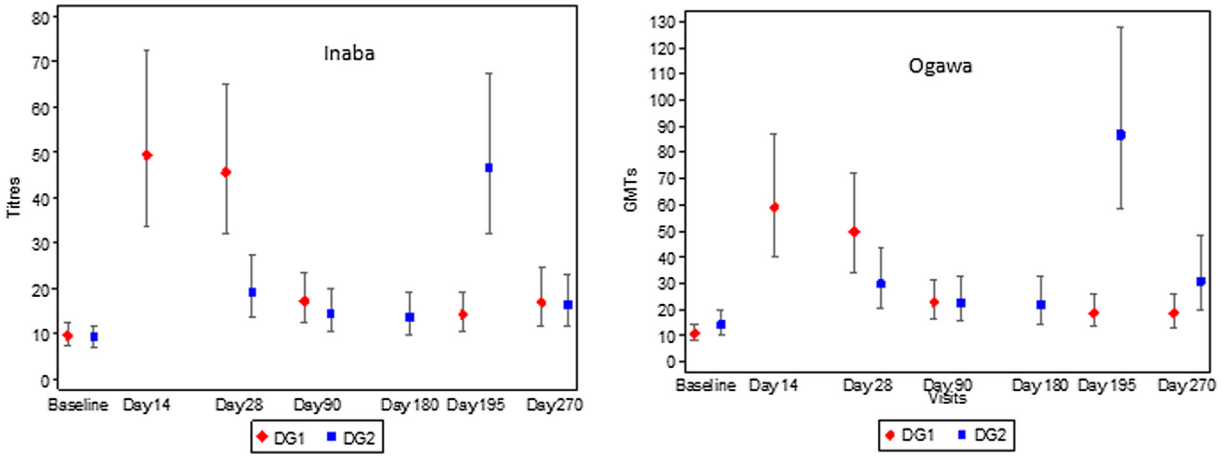

Fig.3. GMT against Inaba and Ogawa by DIGs. Overall trend of the GMT serum titers (Inaba left and Ogawa right) over time by DIG and serotype. **GMT** = Geometric mean titer, **DIG** = Dose Interval Group.

Table 3
Inaba and Ogawa Vibriocidal Titers for Combined Age Groups.

Inaba		**14-day interval DIG1 (n = 80)**	**6-Month Interval DIG2 (n = 76)**	**P-value**
Baseline	GMT(95% CI)	9.6 (7.3, 12.6)	8.8 (6.8, 11.5)	0.66
14 days after first vaccine dose	GMT (95% CI)	50.5 (34.3, 74.4)	N/A	N/A
	GMF rise (95% CI)	5.3 (3.6, 7.7)	N/A	N/A
14 days after second vaccine dose	GMT(95% CI)	45.6 (32.0, 64.9)	47.6 (32.6, 69.3)	0.87
	GMF rise (95% CI)	4.8 (3.4, 6.7)	5.4 (3.8, 7.8)	0.61
	Seroconversion rate (95% CI)	57.5% (46.4%, 68.6%)	65.8% (54.9%, 76.7%)	0.29
3 months after second vaccine dose	GMT (95% CI)	17.4 (12.6, 23.9)	16.0 (11.3, 22.7)	0.72
	GMF rise (95% CI)	1.8 (1.3, 2.4)	1.9 (1.4, 2.5)	0.84
Ogawa		**14-day interval (n = 80)**	**6-month Interval (n = 76)**	**P value**
Baseline	GMT(95% CI)	10.6 (8.0, 14.1)	14.1 (9.8, 20.5)	0.22
14 days after first vaccine dose	GMT (95% CI)	58.1 (38.9,86.6)	N/A	N/A
	GMF rise (95% CI)	5.5 (3.8, 7.8)	N/A	N/A
14 days after second vaccine dose	GMT(95% CI)	49.7 (34.1, 72.3)	87.6 (58.9, 130.4)	0.04
	GMF rise (95% CI)	4.7 (3.4, 6.5)	6.2 (4.3, 8.9)	0.25
	Seroconversion rate (95% CI)	55.0% (43.8% , 66.1%)	63.2% (52.1%, 74.3%)	0.30
3 months after second vaccine dose	GMT (95% CI)	22.4 (16.1, 31.2)	30.1 (19.0, 47.7)	0.30
	GMF rise (95% CI)	2.1 (1.7, 2.6)	2.2 (1.6, 2.9)	0.80

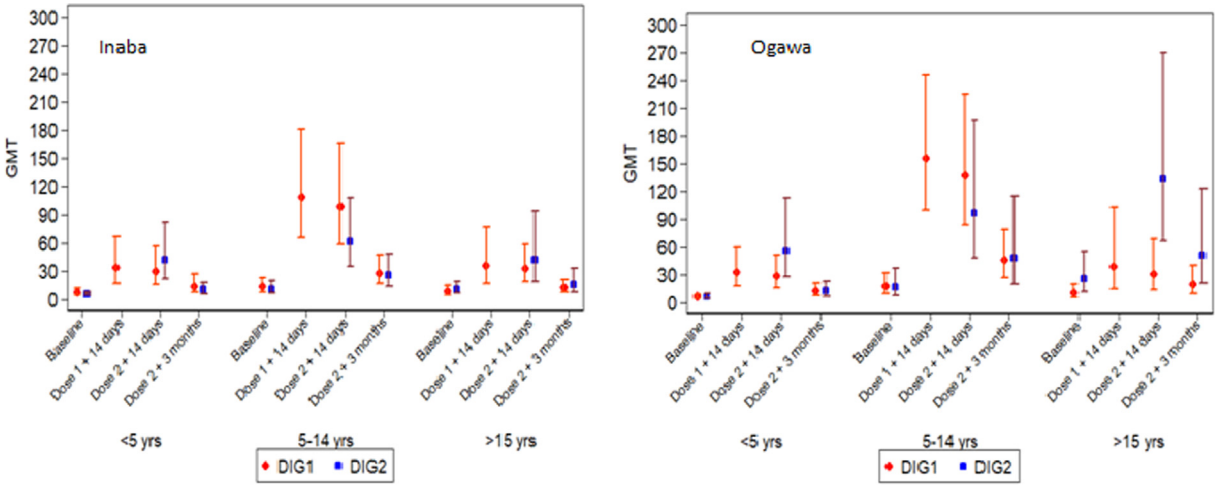

Fig.4. GMTs at baseline and 14 days after the second dose stratified by age group. **GMT** = Geometric mean titer.

dose 1. The studies in both Kolkata and South Sudan were conducted during a time of ongoing cholera infections, and some subjects had likely been primed earlier due to natural exposure. By contrast, the Lukanga Swamps are not endemic but did experience an outbreak a year earlier. However, there were no cases reported during the 12 months preceding, nor during this study so this

study was not confounded by intercurrent infections in the community.

While there were no reports of cholera in the Lukanga Swamps during the study period, an outbreak was occurring in Lusaka during the study period, and it is possible that some persons traveled to Lusaka during the outbreak. It should also be noted that the serotype of the Lusaka strain was Ogawa which might have led to an increase in Ogawa responses in this study relati9ve to Inaba [21]. There were elevated baseline titers in all age groups. However, the proportion of elevated baseline titer did not differ significantly between DIG or by age strata.

Baseline titers were lowest in participants < 5 years of age. The lower GMTs in children < 5 years is in agreement with previous studies from India, Ethiopia and South Sudan [20,22,23]. Since prior receipt of OCV resulted in exclusion from study participation and cholera vibriocidal titers are historically known to drop within twelve months, [24] observing some elevated titers from baseline samples suggests previous exposure to natural cholera infection.

This study demonstrates that the vibriocidal titer 14D post dose 2 is similar regardless of whether OCV was given at a 6-month extended dose-interval or at the standard 2-week dose-interval. This finding is encouraging and may suggest that the interval between the two doses can vary depending on the epidemiological setting. However, this study did not measure protection using these two dose-intervals, and it is understood that the vibriocidal titer does not provide a complete understanding of protection. Epidemiological studies demonstrate a correlation between a higher vibriocidal titer and a lower risk of cholera [25–28]. Clearly, OCV does stimulate an increase in vibriocidal titer, but the titer then falls within a few months while protection persists for several years following receipt of OCV [7,29]. It is clear that a single dose is protective for at least a year for older children and adults, [9,29,30] suggesting that vaccinated individuals are protected with the first dose during the interval before a second dose can be procured and delivered. While a controlled trial to document efficacy of a delayed second dose is unlikely to be undertaken, it may be possible to carry out observational, case-control studies for effectiveness when this strategy is employed.

In practice, when an extended dose interval is implemented, there is a high probability that some of the persons who received the first dose will have moved away or will otherwise not be available at the time the second round is implemented so these persons will receive only one dose. Similarly, others may receive the vaccine during the second round of vaccine distribution who did not receive the first dose. On balance, this strategy may result in more people receiving at least one dose of the vaccine than would have received it if it had been given with the two-week interval. Since a major benefit of OCV is the herd protection it induces, [31,32] the overall protection to the population may increase with the prolonged interval.

This study does not address a question of whether a second dose given at a later time may induce a booster response [33–35]. There is evidence that antibodies, if present in the gut, might bind to antigens from an oral vaccine and interfere with an immune response. If the immune response from the first dose induced a strong local antibody response on day 14, theoretically, this might lessen the response to a second dose administered at this time. Thus, it seems possible that the second dose given 14 days after the first may be less effective than it would have been if it had been delayed until after the local immune response had lessened. This assumption is consistent with studies in Haiti and Bangladesh showing an absence of antibody secreting cell responses after a second dose of OCV at a standard 14-day interval [36]. Unlike methods for identifying a booster response for injected protein vaccines, there are no well-established methods for identifying a booster local intestinal response. Other studies suggest that OCV can induce memory and that a booster response is characterized by a very rapid serological response following OCV [33,34,37]. Our study was not able to evaluate this aspect.

This study had several limitations. While the study found the vibriocidal responses to be very similar in the two groups and we demonstrated non-inferiority by Ogawa serotype, our study did not have a sufficiently large sample size to claim non-inferiority of the Inaba serotype for the extended dose interval [19]. However, it is important to note that these are extremely stringent criteria to demonstrate non-inferiority. Per Donken et al's systematic review of non-inferiority margins, a GMT ratio of 0.5/2.0 is used commonly [19]. Had we stated a priori that we were utilizing the commonly accepted ratio of 0.5/2, our study demonstrates non-inferiority for the extended dose interval. A second limitation included the lack of a D14 serum sample for DIG2, which prevented the ability to directly compare seroconversion rates in the two groups on D14. This blood draw was omitted to minimize the number of blood collections, with the assumption that the responses of DIG1 and DIG2 would be similar since the two groups were randomized. Thirdly, vibriocidal titers did not increase following receipt of OCV in some subjects, but we were not able to investigate causes for the lack of response in these subjects. Reasons for poor responses to oral vaccines are not well understood [38,39].

5. Conclusion

This study demonstrated that vibriocidal GMTs following OCV were similar when comparing the standard 2-week dose-interval to an extended 6-month dose interval. This observation provides reassurance for programs that provide a second dose after a longer period and suggests that a flexible dosing schedule may be appropriate, especially during outbreaks and in situations when stockpile for a second dose is limited or delayed. Observational, case control studies are needed to provide additional evidence of protection when giving the second dose after a longer interval. In addition, studies are needed to determine if a delayed second dose, when the intraluminal antibody titers are reduced, may be superior in boosting intestinal immunological memory.

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CRediT authorship contribution statement

John Mwaba: Methodology, Conceptualization, Writing - original draft. **Caroline Cleopatra Chisenga:** Conceptualization, Writing - review & editing. **Shaoming Xiao:** Data curation, Writing - review & editing. **Harriet Ng'ombe:** . **Elena Banda:** . **Patrick Shea:** Methodology, Writing - review & editing. **Chileshe Mabula-Bwalya:** Writing - review & editing. **Katayi Mwila-Kazimbaya:** Writing - review & editing. **Natasha Makabilo Laban:** Writing - review & editing. **Peter Alabi:** Data curation, Writing - review & editing. **Masuzyo Chirwa-Chobe:** Writing - review & editing. **Michelo Simuyandi:** Methodology, Writing - review & editing. **Jason Harris:** Methodology, Writing - review & editing. **Anita S. Iyer:** . **Samuel Bosomprah:** Data curation, Writing - review & editing. **Paul Scalzo:** Methodology, Writing - review & editing. **Kelsey N. Murt:** Data curation, Writing - review & editing. **Malathi Ram:**

Data curation. **Geoffrey Kwenda:** Writing - review & editing. **Mohammad Ali:** Data curation, Writing - review & editing, Writing - original draft. **David A. Sack:** Project administration, Resources, Writing - review & editing, Writing - original draft. **Roma Chilengi:** Project administration, Writing - review & editing. **Amanda K. Debes:** Project administration, Data curation, Methodology, Writing - review & editing, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.06.034.

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